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16. ABSTRACT

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TRIMETHYLTIN DISRUPTS ACOUSTIC STARTLE RESPONDING IN ADULT RATS*

(Sensory dysfunction; neurotoxicity; behavioral toxicity)

PATRICIA H. RUPPERT, KAREN F. DEAN and LAWRENCE W. REITER

Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC 27711 (U.S.A.)

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SUMMARY

Trimethyltin (TMT) is a limbic-system toxicant which also produces sensory dysfunction in adult animals. In the present experiment, we examined the effects of TMT on the acoustic startle response. Adult male, Long–Evans rats (N = 12/dose) received a single i.p. injection of either 0, 4.0, 5.0 or 6.0 mg/kg TMT hydroxide as the base. The number of responses, latency and peak amplitude of the startle response to a 13 kHz, 120 dB tone were measured 2 h, 2 weeks, and 4 weeks after dosing. For each test session, 10 stimuli were presented at each of three background noise levels (50, 65 and 80 dB). By 2 h after dosing, the number of responses and response amplitude were decreased following 4.0–6.0 mg/kg TMT; these treatment effects persisted through 4 weeks after dosing. Increases in latency were also seen following all dosages of TMT. These data suggest that TMT produces disruption of function within the acoustic-startle pathway.

INTRODUCTION

TMT is a limbic-system neurotoxicant which produces neuronal necrosis within the hippocampus, amygdaloid nucleus and pyriform cortex following acute exposures in adult rats [1, 2]. Behavioral effects include hyperactivity [3, 4] and deficits in learning and memory [5–7]. TMT also produces sensory-system dysfunctions including alterations in visual [8] and somatosensory [9] evoked potentials.

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Abbreviations: IDPN, iminodipropionitrile; TMT, trimethyltin.

The mechanism of this sensory-system toxicity is unknown, but may involve both peripheral and central components. For example, changes in the visual-evoked response indicate both reduced retinal output and increased cortical arousal [8].

TMT also produces swelling of hair cells within the organ of Corti in rats by 24 h after dosing, and hair-cell loss is seen by 30 days after dosing [10]. Preliminary data have indicated decreases in acoustic startle responding [11], reduction or elimination of prepulse inhibition of startle [12], and alterations in brainstem auditory evoked potentials of rats exposed to TMT [13]. In the present study, we determined both dose- and time-effect relationships of acute TMT exposure on the acoustic startle response in adult rats. This response was selected because of its proven usefulness in assessing effects of drugs [14] and brain lesions on sensory-motor reactivity.

METHODS

Male Long-Evans hooded rats (Charles River; Portage, MI), 60 days of age, were housed individually in stainless steel cages measuring 24 × 20 × 18 cm with wire mesh floors. Animals were allowed free access to both Purina Lab Chow and water. The animal room was maintained on a 12 h light-dark cycle beginning at 06.00 h, and testing was conducted during the diurnal period. On day 0, rats (N = 12/group) received a single i.p. injection of either 0 (sterile, 0.9% saline), 4.0, 5.0 or 6.0 mg/kg trimethyltin hydroxide calculated as the base; an injection volume of 1.0 ml/kg was used. This close spacing of dosages was selected because steep dose-response functions have been reported within this range for behavioral effects of TMT [4]. Time-dependent changes in the acoustic startle response were assessed 2 h, 2 weeks and 4 weeks after dosing.

Testing was conducted in 4 sound-attenuated chambers each containing a plastic-framed wire cage (7.6 × 7.6 × 23 cm) mounted on a load cell/force transducer assembly designed to measure vertical force. Two speakers were mounted on the ceiling of each chamber 30 cm above the test cage. One speaker (Motorola, 2″ × 5″ piezo electric tweeter) presented an acoustic stimulus: a 13 kHz, 120 dB, 40 ms tone with a 2.5 ms rise/decay time. A second speaker (special design, full range 16 ohm) delivered background white noise (produced by a Bruel and Kjaer Model 1405 noise generator) at each of 3 intensity levels (50, 65 and 80 dB). These stimulus parameters are similar to those described by Hoffman and Searle [15]. Sound intensity (A scale) was measured within the test chamber using a Bruel and Kjaer sound level meter Model 2209.

Following a 10-min period of adaptation to the chamber at ambient noise level (45 dB), testing was begun. Each rat was presented a total of 30 trials with an inter-trial interval of 20 s; the animals were given 10 trials at each of the 3 background noise levels. Each trial was initiated by presenting the rat with the selected background noise level for 20 s prior to the stimulus presentation. The order of

presentation of the 3 background noise levels was balanced across the session; sequences were randomly generated by computer. The analog response was sampled at a rate of 1 kHz for 72 ms following the stimulus presentation, digitized and converted into grams of force using a calibration constant. The following measures were taken:

(1) *Baseline.* The average force exerted on the platform during the first 10 ms following the stimulus presentation. This value reflects the body weight of the animal.

(2) *Number of responses.* The number of trials per session in which a response occurred (i.e., amplitude exceeded 4 S.D. of the baseline). If the force did not exceed the baseline by 4 S.D., 'no response' was recorded for that trial. Typically, the coefficient of variability was 1% of the baseline; therefore, a change in baseline of approx. 4% or greater was recorded as a startle response. Only trials in which a response occurred were used to calculate session means for the latency to onset and amplitude of the response (i.e., values of zero were *not* included for 'no response' trials).

(3) *Latency to onset.* The interval from the stimulus onset to the time (ms) when the force exerted on the platform was 4 S.D. above the baseline.

(4) *Amplitude.* The maximum force exerted on the platform during the response, corrected for (i.e., minus) the baseline.

For the number of responses, a proportion of the total possible responses (pooled across background noise levels) was obtained for each animal. The arcsin of the proportion was analyzed by multivariate repeated-measures analyses [16], using time after dosing as the within-subjects factor. Amplitude and latency of responses were averaged across trials at each background noise level and analyzed using multivariate repeated-measures analyses with background noise and time after dosing as within-subjects factors. As a measure of sensitization, differences between the amplitudes of the startle response at the 50 and 80 dB background noise levels were calculated for each animal. These values were analyzed by repeated-measures ANOVA using time after dosing and background noise as within-subjects factors. When significant treatment interactions were found, simple main effects tests (ANOVA) were conducted. Post-hoc comparisons were made using Tukey's (a) test [17]. Analyses were performed using programs on the Statistical Analysis System (SAS, 1982) and the Biomedical Data Program (BMDP, 1981).

RESULTS

The proportion of responses was decreased by TMT at all times after dosing [$F(9,102) = 5.39$; $P < 0.0001$]. Animals receiving all dosages of TMT made significantly fewer responses than controls (Fig. 1A).

Latency to the onset of the response was increased by TMT [$F(3,42) = 6.16$, $P < 0.0014$]. All dosages of TMT significantly increased latency (Fig. 1B). There was

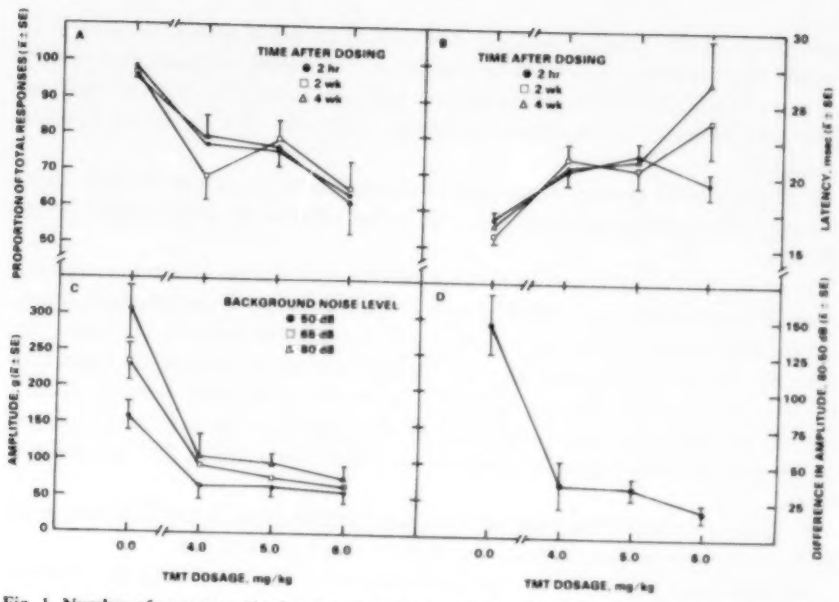

Fig. 1. Number of responses (A), latency to onset (B), amplitude (C), and amplitude difference (D) for a 13 kHz, 120 dB stimulus in controls and TMT-treated rats. All values are mean ± SE. All dosages of TMT produced a decrease in the number of trials in which the animals responded (A), an increase in latency to respond (B), a decrease in response amplitude (C), and reduced amplitude facilitation by increasing levels of background noise (D).

also a time by treatment interaction [$F(6,82) = 2.88$, $P < 0.0134$]; latency increased over time for the 6.0 mg/kg TMT-treated animals only [$F(2,41) = 11.06$, $P < 0.0001$]. Latency decreased as a function of background noise [$F(2,41) = 16.08$, $P < 0.0001$], but there was no interaction with treatment.

Response amplitude was reduced by TMT at all times after dosing [$F(3,42) = 13.95$, $P < 0.0001$]. There was an increase in amplitude over time [$F(2,41) = 3.90$, $P < 0.0282$] but since there was no interaction of treatment with time, data have been collapsed across times. Startle amplitudes were significantly reduced in all TMT-treatment groups (Fig. 1C). There was an effect of background noise by treatment interaction [$F(6,82) = 6.39$, $P < 0.0001$]. This interaction suggests a difference in sensitization to background noise levels as a function of treatment. An analysis of the change in amplitude from the 50 to 80 dB background noise levels indicated a significant effect of treatment at all times after dosing [$F(3,44) = 14.41$, $P < 0.0001$]. All dosages of TMT significantly reduced sensitization (Fig. 1D).

DISCUSSION

TMT produced both acute and persistent deficits in auditory startle responding

following a single exposure in adult animals: following all dosages of TMT the number of responses, response amplitude and sensitization by background noise were decreased while response latency was increased. Effects of TMT on acoustic startle were seen at lower dosages than those producing the TMT syndrome [18] or behavioral changes associated with hippocampal dysfunction [3–7]. These deficits were produced within 2 h of dosing and recovery was not seen by 4 weeks after dosing.

The most dramatic treatment effect produced by TMT was the decrease in the number of responses to the 120 dB tone stimulus. Although hippocampal lesions characterize TMT toxicity [1, 2], hippocampal lesions either have no effect [19, 20] or increase startle responsiveness [21]. Likewise, lesions of the amygdala do not disrupt startle responding [19]. While drugs which affect a variety of neurotransmitter systems can modulate the amplitude of the startle response, none of these drug treatments abolish startle or even depress it markedly [14]. However, electrolytic lesions within the primary acoustic startle circuit also abolish or markedly attenuate startle responding [22]. A similar decrease in the number of startle responses was reported in rats following acute exposure to IDPN [23]. IDPN produces axonal lesions in the nucleus reticularis pontine caudalis, as well as damage to the nuclei of the eighth nerve and motor neurons [24]. Therefore, both electrolytic and neurotoxic lesions of the primary auditory startle circuit can produce a decrease in the number of startle responses.

The startle reflex can be modified by stimulus factors, including the level of background noise [15]. Sensitization, an increase in amplitude and decrease in latency of the response produced by increasing levels of background noise, was seen in control animals. Following all dosages of TMT, the sensitization effect of background noise on amplitudes but not latencies was significantly reduced. While the neural mechanisms underlying sensitization are not understood, these data suggest a differential effect on amplitude and latency. However, this lack of sensitization for amplitudes at higher dosages could represent a 'floor' effect, so that further amplitude decreases were classified as non-responses. For example, while the reduction in amplitude was comparable at all dosages of TMT, the number of responses was dose-related. Also, sensitization effects on latency were maintained at all dosages of TMT, while latencies to onset were increased. Since the neural circuit for the startle reflex probably involves only 5 synapses plus the neuromuscular junction [22], increases in latency produced by TMT are quite significant. Further studies at lower dosages of TMT are needed to determine the threshold for these effects.

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